A new transcription element in the JC virus enhancer

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The enhancer of JC virus restricts its gene expression to the brain. In a previous study, we demonstrated an enhancer element, nuclear factor I (NFI) motif, in the middle of the enhancer. Here, we demonstrate that the NFI motif is a tissue-specific element. We further present a new tissue-specific element, SacI motif, just upstream from the NFI motif. These motifs showed transcriptional enhancement both in vitro and in vivo and acted upon a heterologous adenovirus major late promoter. DNase I footprint analyses demonstrated that the SacI motif bound to a brain nuclear factor, and that its binding region overlapped with the NFI motif. Gel shift experiments with the SacI motif revealed that the populations of SacI motif factors in the brain and HeLa cell extracts were different. Together with our previous findings about tissue-specific NFI-like factor(s), cooperation of NFI motif and SacI motif factors may be required for the strong brain specificity of the viral gene expression.

JC virus (JCV) is a common human polyomavirus which causes demyelinating diseases and/or brain tumours among rodents and primates (Walker et al., 1973; Padgett & Walker, 1976; Beckmann et al., 1982). As JCV gene expression is restricted to the brain, this virus is a good tool for studying brain-specific gene expression. It has been suggested that regulation of JCV gene expression is mediated through a viral enhancer which consists of a 98 bp tandem repeat (Kenney et al., 1984; Small et al., 1986). Previous in vivo and in vitro analyses demonstrated that the JCV enhancer has multiple cis-acting transcription elements (Padgett et al., 1977; Martin et al., 1983; Tada et al., 1989). Khalili et al. (1988) have suggested that a short stretch of DNA between map positions (m.p.) 37 and 62 of JCV includes essential sequences for brain-specific viral gene expression. Several groups have demonstrated that a nuclear factor I (NFI) motif is present in this region and that the NFI motif actually functions during JCV DNA transcription (Tamura et al., 1988; Amemiya et al., 1989). However, transcription elements and their cognate factors in this region have not yet been thoroughly characterized. In this study, we have identified another tissue-specific transcription element in the critical region of the JCV enhancer which overlaps with the NFI motif.

Although the JCV enhancer consists of a tandem 98 bp repeat (two enhancer units), we used viral DNA which contained a single enhancer unit in this study to simplify the analyses (Fig. 1). A PvuII (5112 m.p.)/HindIII (268 m.p.) fragment of JCV DNA carrying one enhancer unit was inserted into pBR322 to construct pJC1B. pJC1B was transcribed in mouse brain nuclear extract and specific transcripts from the viral early promoter were analysed by S1 nuclease mapping (Tamura et al., 1988, 1989) (Fig. 2). Previous studies (Tamura et al., 1988) demonstrated that in vitro transcription of the two enhancer unit-carrying DNA was initiated from the wrong position (91 m.p.) for unknown reasons (Fig. 1a). In this experiment, however, we found that the in vitro start site of pJC1B DNA (indicated in Fig. 2) was analogous to a region found in vivo (5122 m.p.). Primer extension analysis indicated that in vitro transcripts were initiated from 5121 m.p. (data not shown) (Fig. 1a). HeLa cells have been used as representative non-permissive cells for JCV gene expression. We transcribed pJC1B DNA in HeLa cell extracts (Fig. 2) and found that they were almost inactive in transcription of the viral early gene under conditions in which the adenovirus major late promoter (MLP), used as an external control, was equally transcribed (Fig. 2b, lane 1). This suggests that the in vitro transcription found using mouse brain nuclear extracts is of significance for use in the study of the brain specificity of JCV gene expression.

To investigate the sequences required around the NFI motif...
motif for transcription activation, we constructed mutant DNAs (Fig. 1). There is a unique Sacl site at 57 m.p., just upstream of the NFI motif. By the deletion of 5 bp around this site (57 to 61 m.p.) in the pJC1B construct, pJC1BS DNA was formed. An NFI motif mutant, pJC1BN, was also made which has base substitutions at 48, 49 and 51 m.p. Both pJC1BS and pJC1BN were transcribed in mouse brain nuclear extract (Fig. 2). As shown in Fig. 2b (lane 10), the transcription signal from pJC1BS DNA was weaker than that from pJC1B DNA. These results suggested that sequences around the Sacl site contain a positive transcription element which functions in vitro. Similar experiments were carried out using pJC1BN DNA (Fig. 2a), and the results indicated that the NFI motif functioned in vitro as previously observed, using two enhancer unit-carrying JCV DNA (Tamura et al., 1988). The single enhancer-carrying JCV DNAs were transcribed in non-specific HeLa cell extracts. We found that the mutations in both pJC1BN and pJC1BS DNA had little effect on transcription in HeLa cell extracts (Fig. 2, lanes 6 and 13). Consequently, the NFI motif, and sequences around the Sacl site, were found to be tissue-specific transcription elements functional in vitro. We designated the sequences around the Sacl site as the Sacl motif. To analyse enhancer function more sensitively, we constructed a series of chimeric promoters containing both MLP and JCV sequences (Fig. 1b). Chimeric templates were transcribed in brain nuclear extract. As shown in Fig. 3, strong MLP transcription signals were obtained. Compared to pML, pML/JC and pML/JCN both gave stronger signals. However, the amount of transcript formed by pML/JCN, pML/JCNi and pML/JCS was obviously lower than that formed by pML/JC, and seemed to be similar to that from pML.
Short communication

Fig. 3. The JCV enhancer acts upon the MLP. 380 ng of pML (lane 1), pML/JC (lane 2), pML/JCi (lane 3), pML/JCN (lane 4), pML/JCni (lane 5) and pML/JCS (lane 6) DNA were transcribed in brain nuclear extract and MLP transcripts (arrowhead) were compared. pML/JCN and pML/JCS contained JCV DNA sequences of pJC1BN and pJC1BS, respectively, similarly to pML/JC. However, pML/JCi and pML/JCni contained the viral sequences of pJC1B and pJC1BN DNA respectively inserted into the EcoRI site of pML in an inverted orientation. Lane M contains pBR322/MspI markers.

These results suggested that both the NFI and SacI motifs functioned as enhancer elements, even on a heterologous promoter.

We carried out in vivo transcription experiments using primary cultured rat brain cells and the luciferase assay (de Wet et al., 1987) (Fig. 4). The viral sequence from PvuII to HindIII in pJC1B, pJC1BN or pJC1BS DNA was inserted into the unique HindIII site of the luciferase expression vector, pSVOAL, in the normal orientation, and transiently expressed intracellular luciferase activity was measured. Since experiments were carried out in triplicate, and the standard deviations were small (data not shown), transfection efficiencies were probably similar in all experiments. pSVL/J DNA carrying an intact single enhancer unit yielded high luciferase activity. pSVL/JN and pSVL/JS DNA yielded lower enzyme activities than pSVL/J. These results show that the JCV early promoter/enhancer was active in vivo.

Enhancer motifs naturally function in virus gene expression.

We performed a DNase I footprint experiment to analyse the trans-acting factor of the SacI motif (Fig. 5a, b). We found strong protections in the NFI motif and pseudo-NFI (ψ-NFI) motif of pJC1B DNA as previously reported (Tamura et al., 1988). In this study, we found a further protected site on sequences just upstream from the NFI motif. This footprint fused with the NFI motif. The precise region of the NFI motif footprint had already been determined (Tamura et al., 1988). When we used pJC1BN DNA, part of the NFI motif footprint disappeared, leaving protection of the SacI motif, the footprint of which became slightly weaker. Footprint analysis using pJC1BS DNA revealed no protection of sequences from 56 to 63 m.p., but the NFI motif was protected (Fig. 5b). Since there was a correlation
between the promoter activity and the binding of the SacI motif, we suggest that the SacI motif binding factor is a positive transcription factor. HeLa cell extracts also exhibited a similar footprinting pattern for the SacI motif (data not shown). We carried out gel shift assays to compare the composition of SacI motif factors between extracts (Fig. 5). The brain nuclear extract yielded one retarded band (C1), whereas HeLa cell extract gave rise to two bands (C1 and C2), both of which were confirmed as specific complexes (data not shown). Amounts of the C1 complex in HeLa cell extract were lower than those in the brain nuclear extract. These results revealed that the composition of SacI motif factors was characteristic for different tissues and cells.

The JCV enhancer from 37 to 62 m.p. is considered to be essential for brain-specific transcription (Khalili et al., 1988). Together with a previous study (Tamura et al., 1988), we identified NFI and SacI motifs as tissue-specific cis-elements in this region and trans-acting factors for each motif were also identified. DNase I footprint, using mutant sequences, specified the position of the SacI motif (43 to 63 m.p.), which overlapped the NFI motif. These two motifs may play an essential role in the JCV life cycle, as they function in vivo and naturally occurring JCV variants always retain these two motifs (Martin et al., 1985). We have already demonstrated heterogeneous populations of NFI-like factors among tissues, and suggested the presence of tissue-specific NFI-like factors (Aoyama et al., 1990). Here, we found that the SacI motif factor also exhibited a different composition between brain and HeLa cell extracts. We suggest that the presence of two tissue-specific elements and their cognate factors are required for the absolute brain specificity of the JCV enhancer function. We suggest that a factor in the C1 complex is a positive transcription factor which is enriched in the brain. It may be possible that the C2 complex-forming factor in HeLa cells can function as a transcriptional repressor. It is interesting that two tissue-specific elements are located in close proximity to each other. We noted that the SacI motif footprints of pJC1BN were slightly weaker than those of pJC1B, and that the protection patterns of the SacI motif on pJC1B and pJC1BN were not exactly the same (Fig. 5a), suggesting a protein–protein interaction between two binding factors. Cooperative transcriptional activation by these two factors can be suggested.

We wish to thank Mrs Y. Ohyu for technical assistance. This work was supported by Grants-in-Aid for Scientific Research on Priority Areas from the Japanese Ministry of Education, Science and Culture.

References


